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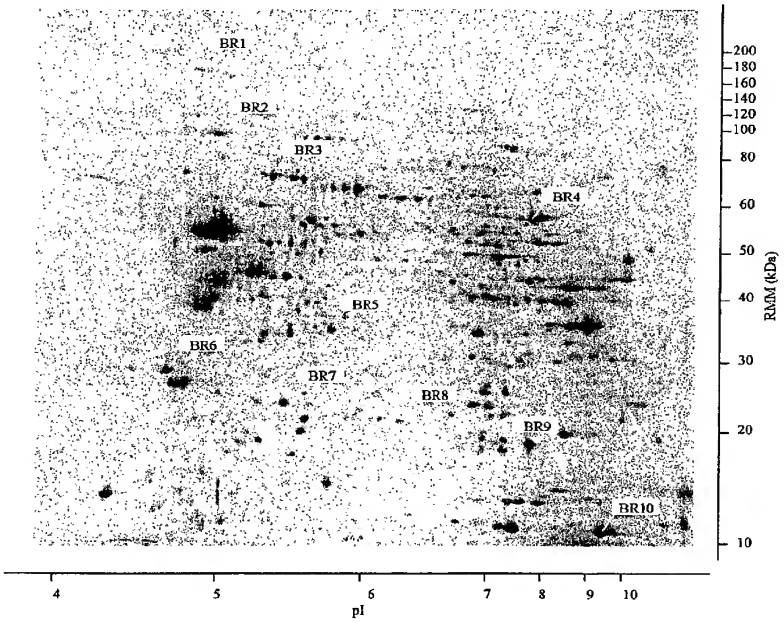

(57) Abstract: The present invention provides a protein (ADPI-41) identified in brain tissue, compositions comprising the protein, including vaccines and antibodies, which are immunospecific for the protein. The use of the protein in the diagnosis, screening, treatment and prophylaxis of Alzheimer's and other diseases is also provided.

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PROTEINS

The present invention relates a human protein, named herein as ADPI-41, which is differentially expressed in several areas of the brain in Alzheimer's disease. It also relates to compositions comprising the protein, including antibodies which are immunospecific for the protein, and the use of these in diagnosis, prophylaxis and treatment.

Alzheimer's disease (AD) is an increasingly prevalent form of neurodegeneration that accounts for approximately 50-60 % of the overall cases of dementia among people over 65 years of age. It currently affects an estimated 15 million people worldwide and, owing to the relative increase of elderly people in the population, its prevalence is likely to increase over the next 2 to 3 decades. Alzheimer's disease is a progressive disorder with a mean duration of around 8.5 years between onset of clinical symptoms and death. Death of pyramidal neurons and loss of neuronal synapses in brains regions associated with higher mental functions results in the typical symptomology, characterized by gross and progressive impairment of cognitive function (Francis *et al.*, 1999, *J. Neurol. Neurosurg. Psychiatry* **66**:137-47). Currently, a diagnosis of Alzheimer's disease requires a careful medical history and physical examination; a detailed neurological and psychiatric examination; laboratory blood studies to exclude underlying metabolic and medical illnesses that masquerade as AD; a mental status assessment and formal cognitive tests; and a computed tomographic scan or magnetic resonance image of the brain (Growdon, JH., 1995, Advances in the diagnosis of Alzheimer's disease. In: Iqbal, K., Mortimer, JA., Winblad, B., Wisniewski, HM eds *Research Advances in Alzheimer's Disease and Related Disorders.* New York, NY: John Wiley & Sons Inc. 1995:139-153). Due to the time consuming nature of these tests, their expense, and their inconvenience to patients, it would be highly desirable to measure a substance or substances in body samples, such as samples of tissue, cerebrospinal fluid (CSF), blood or urine, that would lead to a positive diagnosis of Alzheimer's disease or that would help to exclude AD from the differential diagnosis.

Since the CSF bathes the brain, changes in its protein composition may most accurately reveal alterations in brain protein expression patterns that are causatively or diagnostically linked to the disease.

Current candidate biomarkers for Alzheimer's disease include: (1) mutations in presenilin 1 (PS1), presenilin 2 (PS2) and amyloid precursor protein (APP) genes; (2) the detection of alleles of apoplipoprotein E (ApoE); and (3) altered concentrations of amyloid β-peptides (Aβ), tau protein, and neuronal thread protein (NTP) in the CSF. See, *e.g.*, *Neurobiology of Aging* **19**:109-116 (1998) for a review. Mutations in PS1, PS2 and APP genes are indicative of early-onset familial Alzheimer's disease. However, early-onset familial Alzheimer's disease is relatively rare; only 120 families worldwide are currently known to carry deterministic mutations (*Neurobiology of Aging* **19**:109-116 (1998)). The detection of the e4 allele of ApoE has been shown to correlate with late-onset and sporadic forms of Alzheimer's disease. However, e4 alone cannot be used as a biomarker for Alzheimer's disease since e4 has been detected in many individuals not suffering from Alzheimer's disease and the absence of e4 does not exclude Alzheimer's disease (*Neurobiology of Aging* **19**:109-116 (1998)).

A decrease in the Aβ peptide Aβ42 and an increase in tau protein in the CSF of Alzheimer's disease have been shown to correlate with the presence of Alzheimer's disease (*Neurobiology of Aging* **19**:109-116 (1998)). However, the specificity and sensitivity of Aβ42 and tau protein as biomarkers of Alzheimer's disease are modest. For example, it has been difficult to determine a cutoff level of CSF tau protein that is diagnostically informative. Also, elevated levels of NTP in the CSF of postmortem subjects has been shown to correlate with the presence of Alzheimer's disease (*Neurobiology of Aging* **19**:109-116 (1998)). Therefore, a need exists to identify sensitive and specific biomarkers for the diagnosis of Alzheimer's disease, as well as other neuropsychiatric or neurological disorders, in living subjects.

The present invention discloses a protein isoform, referred to ADPI-41, which has been found to be differentially expressed in Alzheimer's disease.

Thus, in a first aspect, the present invention provides a substantially pure, isolated or recombinant polypeptide which includes the sequence YIYDSAFHPDTGEK.

Preferably, the polypeptide includes the sequence:
ANHFFTVTDPRNILLTNEQLESARKIVHDYRQGIVPPGLTENELWRAKYIYDSA
FHPDTGEKMILIGRMSAQVPMNMTITGCMMTFYRTTPAVLFWQWINQSFNAV
VNYTNR.

More preferably, the polypeptide:

a) comprises or consists of the amino acid sequence shown in Figure 2b or 3b;

b) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence shown in Figure 2b or 3b; or

c) is a fragment of a polypeptide as defined in a) or b) above, which is at least ten amino acids long.

The present invention will be better understood from a consideration of the ensuing detailed description. Reference is made to the accompanying drawings, in which:

Figure 1 is an image obtained from 2-dimensional electrophoresis of normal tissue, which has been annotated to identify ten landmark features, designated BR1 to BR12;

Figure 2 shows nucleic acid sequence of ADPI-41 (Figure 2a) and the corresponding amino acid sequence (Figure 2b) where the tryptic peptides identified by mass spectrometry are underlined, motifs conserved in mouse sideroflexin 1are boxed and sequences shaded in grey represent the predicted transmembrane domains; and

Figure 3 shows the nucleic acid sequence (Figure 3a) and the corresponding amino acid sequence (Figure 3b) of a splice variant identified for ADPI-41. The protein sequence (Figure 3b) shows in bold the amino acids unique to this splice variant, the tryptic digest peptides identified by mass spectroscopy are underlined, motifs conserved in mouse sideroflexin 1 are boxed and sequences shaded in grey represent the predicted transmembrane domains.

ADPI-41 has been isolated by 2D-electrophoresis and shown to have an apparent molecular weight of 32806 Da and apparent pI of 9.84, measured as described in WO 98/23950. When samples are run in duplicate or a higher number of replicates, variation in the measured mean pI of ADPI-41 is typically less than 3% and variation in the measured mean MW of ADPI is typically less than 5%.

It has subsequently been characterised by mass spectrometry and, as a result of database searching, the following amino acid sequences of tryptic digest peptides were determined from matches to conceptual translations of ESTs: BE298534, AI014241, AV655958, AA568689, AW796078, AA782417 BF126487, BG388906 and BG577432. These sequences have a very high similarity to tryptic digest peptides in the mouse sideroflexin 1 protein (from mitochondria, accession number 15147224, accessible at http://www.ncbi.nlm.nih.gov/entrez/), identified with a subsequent Blast search (http://www.ncbi.nlm.nih.gov/blast).

ADPI-41 has been identified in brain tissue homogenate of human subjects, and been shown to be significantly differentially expressed in the brain tissue of subjects having Alzheimer's disease as compared with the brain tissue of subjects free from Alzheimer's disease. Accordingly, it finds utility as a marker for a neuropsychiatric or neurological disorder, i.e. a disturbance in structure or function of the central nervous system resulting from developmental abnormality, disease, injury or toxin, or a mental illness arising from the same. Such disorders include, without limitation, dementing

illnesses such as Alzheimer's disease, vascular dementia and Lewy body dementia, as well as schizophrenia, Parkinson's disease, multiple sclerosis, and depression.

Thus, quantitative detection of ADPI-41 in brain biopsies can be used to diagnose a neuropsychiatric or neurological disorder, determine the progression of such a disorder and/or monitor the effectiveness of a therapy for such a disorder.

Thus, in a second aspect, the invention provides a method of screening for and/or diagnosis of a neuropsychiatric or neurological disorder in a subject and/or monitoring the effectiveness of a neuropsychiatric or neurological disorder therapy, which method comprises the step of detecting and/or quantifying the amount of a polypeptide of the first aspect in a biological sample obtained from said subject.

In a third aspect, the present invention provides a method for the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder in a subject, which comprises administering to said subject a therapeutically effective amount of at least one polypeptide as defined in the first aspect of the invention.

In a fourth aspect, the present invention provides the use of at least one polypeptide as defined in the first aspect of the invention in the preparation of a medicament for use in the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder.

The subject may be a mammal and is preferably a human, although monkeys, apes, cats, dogs, cows, horses and rabbits are within the scope of the present invention.

The polypeptides or fragments thereof of the invention may be provided in isolated or recombinant form. As used herein, a polypeptide is "isolated" when it is present in a preparation that is substantially free of contaminating proteins, i.e. a preparation in which no more than 25% (preferably no more than 10% or 5%, more preferably less than 1%) of the total protein present is contaminating protein(s). A contaminating

protein is a protein having a different amino acid sequence from that of the isolated polypeptide.

In order to more fully appreciate the present invention, polypeptides within the scope of a)-c) above will now be discussed in greater detail.

Polypeptides within the scope of a)

A polypeptide of the invention, especially one within the scope of a), may have an additional N-terminal and/or an additional C-terminal amino acid sequence relative to these sequences.

Additional N-terminal or C-terminal sequences may be provided for various reasons. Techniques for providing such additional sequences are well known in the art.

Additional sequences may be provided in order to alter the characteristics of a particular polypeptide. This can be useful in improving expression or regulation of expression in particular expression systems. For example, an additional sequence may provide some protection against proteolytic cleavage. This has been done for the hormone Somatostatin by fusing it at its N-terminus to part of the β galactosidase enzyme (Itakwa *et al.*, *Science* 198: 105-63 (1977)).

Additional sequences can also be useful in altering the properties of a polypeptide to aid in identification or purification. For example, a fusion protein may be provided in which a polypeptide is linked to a moiety capable of being isolated by affinity chromatography. The moiety may be an antigen or an epitope and the affinity column may comprise immobilised antibodies or immobilised antibody fragments which bind to said antigen or epitope (desirably with a high degree of specificity). The fusion protein can usually be eluted from the column by addition of an appropriate buffer. Fusions with localisation-

reporter proteins, such as the Green Fluorescent Protein (U.S. Patent Nos. 5,625,048, 5,777,079, 6,054,321 and 5,804,387) or the DsRed fluorescent protein (Matz *et al* (1999) *Nature Biotech.* 17:969–973) are specifically contemplated.

Additional N-terminal or C-terminal sequences may, however, be present simply as a result of a particular technique used to obtain a polypeptide and need not provide any particular advantageous characteristic to the polypeptide, such as an N-terminal methionine where a eukaryotic protein is produced in a bacterial expression system. Such polypeptides are within the scope of the present invention.

Whatever additional N-terminal or C-terminal sequence is present, it is preferred that the resultant polypeptide should exhibit the immunological or biological activity of the polypeptide comprising the amino acid sequences shown in either Figures 2b or 3b.

Polypeptides within the scope of b)

Turning now to the polypeptides defined in b) above, it will be appreciated by the person skilled in the art that these polypeptides are variants of the polypeptide given in a) above. Such variants preferably exhibit the immunological or biological activity of the polypeptide comprising or consisting of the amino acid sequence shown in either Figures 2b or 3b.

Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the protein's biological or immunological function.

The skilled person will appreciate that various changes can often be made to the amino acid sequence of a polypeptide which has a particular activity to produce variants (sometimes known as "muteins") having at least a proportion of said activity, and preferably having a substantial proportion of said activity. Such variants of the polypeptides described in a) above are within the scope of the present invention and are discussed in greater detail below. They include allelic and non-allelic variants.

An example of a variant of the present invention is a polypeptide as defined in a) above, apart from the substitution of one or more amino acids with one or more other amino acids. The skilled person is aware that various amino acids have similar properties. One or more such amino acids of a polypeptide can often be substituted by one or more other such amino acids without eliminating a desired activity of that polypeptide.

Thus, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions, it is preferred that glycine and alanine are used to substitute for one another (since they have relatively short side chains) and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic).

Other amino acids that can often be substituted for one another include:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Substitutions of this nature are often referred to as "conservative" or "semi-conservative" amino acid substitutions.

Amino acid deletions or insertions may also be made relative to the amino acid sequence given in a) above. Thus, for example, amino acids which do not have a substantial effect on the biological and/or immunological activity of the polypeptide, or at least which do not eliminate such activity, may be deleted. Such deletions can be advantageous since the overall length and the molecular weight of a polypeptide can be reduced whilst still retaining activity. This can enable the amount of polypeptide required for a particular purpose to be reduced – for example, dosage levels can be reduced.

Amino acid insertions relative to the sequence given in a) above can also be made. This may be done to alter the properties of a polypeptide used in the present invention (e.g. to assist in identification, purification or expression, as explained above in relation to fusion proteins).

Amino acid changes relative to the sequence given in a) above can be made using any suitable technique e.g. by using site-directed mutagenesis (Hutchinson *et al.*, 1978, *J. Biol. Chem.* 253:6551).

It should be appreciated that amino acid substitutions or insertions within the scope of the present invention can be made using naturally occurring or non-naturally occurring amino acids. Whether or not natural or synthetic amino acids are used, it is preferred that only L- amino acids are present.

Whatever amino acid changes are made (whether by means of substitution, insertion or deletion), preferred polypeptides of the present invention have at least 50% sequence identity with a polypeptide containing the sequence YTYDSAFHPDTGEK or ANHFFTVTDPRNILLTNEQLESARKIVHDYRQGIVPPGLTENELWRAKYTYDSA FHPDTGEKMILIGRMSAQVPMNMTITGCMMTFYRTTPAVLFWQWINQSFNAV VNYTNR, or as defined in a) above, more preferably the degree of sequence identity

is at least 75%. Sequence identities of at least 90%, 95%, 96%, 97%, 98% or at least 99% are most preferred.

The percent identity of two amino acid sequences or of two nucleic acid sequences is determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (*i.e.*, % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilised as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilising BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov.

Another example of a mathematical algorithm utilised for the comparison of sequences is the algorithm of Myers & Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the CGC sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis & Robotti (1994) *Comput. Appl. Biosci., 10* :3-5; and FASTA described in Pearson & Lipman (1988) *Proc. Natl. Acad. Sci. 85*:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

Where high degrees of sequence identity are present there will be relatively few differences in amino acid sequence. Thus for example they may be less than 20, less than 10, or even less than 5 differences.

Polypeptides within the scope of c)

As discussed *supra*, it is often advantageous to reduce the length of a polypeptide, provided that the resultant reduced length polypeptide still has a desired activity or can give rise to useful antibodies. Feature c) of the present invention therefore covers fragments of polypeptides a) or b) above.

The skilled person can determine whether or not a particular fragment has activity using the techniques disclosed above. Preferred fragments are at least 10 amino acids long. They may be at least 20, at least 50 or at least 100 amino acids long.

The skilled person will appreciate that, for the preparation of a polypeptide of the invention, the preferred approach will be based on recombinant DNA techniques. In addition, nucleic acid molecules encoding the polypeptides or fragments thereof may be used in their own right. Thus, in a fifth aspect, the invention provides an isolated or recombinant nucleic acid molecule which encodes a polypeptide of the present invention.

Preferably, the nucleic acid molecule:

a) comprises or consists of the DNA sequence shown in Figure 2a or 3a or its RNA equivalent;

b) has a sequence which is complementary to the sequences of a);

c) has a sequence which codes for the same polypeptide as the sequences of a) or b);

d) has a sequence which shows substantial identity with any of those of a), b) and c); or

e) has a sequence which codes for a derivative or fragment of an amino acid molecule shown either in Figures 2b or 3b.

In sixth aspect, the invention provides a method of screening for and/or diagnosis of a neuropsychiatric or neurological disorder in a subject and/or monitoring the effectiveness of a neuropsychiatric or neurological disorder therapy, which method comprises the step of detecting and/or quantifying the amount of a nucleic acid of the invention in a biological sample obtained from said subject.

In a seventh aspect, the present invention provides a method for the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder in a subject, which comprises administering to said subject a therapeutically effective amount of at least one nucleic acid of the invention.

In an eighth aspect, the present invention provides the use of at least one nucleic acid of the invention in the preparation of a medicament for use in the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder.

These nucleic acid molecules are now discussed in greater detail.

It is preferred if sequences which show substantial identity with any of those of a), b) and c) have e.g. at least 50%, at least 75% or at least 90%, 95%, 96%, 97%, 98% or 99% sequence identity.

The polypeptides used in the present invention can be coded for by a large variety of nucleic acid molecules, taking into account the well-known degeneracy of the genetic code. All of these molecules are within the scope of the present invention. They can be inserted into vectors and cloned to provide large amounts of DNA or RNA for further study. Suitable vectors may be introduced into host cells to enable the expression of polypeptides used in the present invention using techniques known to the person skilled in the art.

The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may be in isolated, recombinant or chemically synthetic form.

Techniques for cloning, expressing and purifying proteins and polypeptides are well known to the skilled person. DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2nd Edition*, Cold Spring Harbour Laboratory Press (1989); in Old & Primrose *Principles of Gene Manipulation* 5th Edition, Blackwell Scientific Publications (1994); and in Stryer, *Biochemistry* 4th Edition, W H Freeman and Company (1995). Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

Normally the DNA construct will be inserted into a vector, which may be of phage or plasmid origin. Expression of the protein is achieved by the transformation or

transfection of the vector into a host cell, which may be of eukaryotic or prokaryotic origin. Such vectors and suitable host cells form aspects of the present invention.

Knowledge of the nucleic acid structure can be used to raise antibodies and for gene therapy. Techniques for this are well known by those skilled in the art, as discussed in more detail herein.

By using appropriate expression systems, polypeptides of the present invention may be expressed in glycosylated or non-glycosylated form. Non-glycosylated forms can be produced by expression in prokaryotic hosts, such as *E. coli.*

Polypeptides comprising N-terminal methionine may be produced using certain expression systems, whilst in others the mature polypeptide will lack this residue.

Preferred techniques for cloning, expressing and purifying a polypeptide used in the present invention are summarised below:

Polypeptides may be prepared natively or under denaturing conditions and then subsequently refolded. Baculoviral expression vectors include secretory plasmids (such as pACGP67 from Pharmingen), which may have an epitope tag sequence cloned in frame (e.g. myc, V5 or His) to aid detection and allow for subsequent purification of the protein. Mammalian expression vectors may include pCDNA3 and pSecTag (both Invitrogen), and pREP9 and pCEP4 (invitrogen). *E.coli* systems include the pBad series (His tagged - Invitrogen) or pGex series (Pharamacia).

In addition to nucleic acid molecules coding for polypeptides used in the present invention, referred to herein as "coding" nucleic acid molecules, the present invention also includes nucleic acid molecules complementary thereto. Thus, for example, both strands of a double stranded nucleic acid molecule are included within the scope of the

present invention (whether or not they are associated with one another). Also included are mRNA molecules and complementary DNA Molecules (e.g. cDNA molecules).

Nucleic acid molecules which can hybridise to any of the nucleic acid molecules discussed above are also covered by the present invention. Such nucleic acid molecules are referred to herein as "hybridising" nucleic acid molecules. Hybridising nucleic acid molecules can be useful as probes or primers, for example.

Desirably such hybridising molecules are at least 10 nucleotides in length and preferably are at least 25 or at least 50 nucleotides in length. The hybridising nucleic acid molecules preferably hybridise to nucleic acids within the scope of (a), (b), (c), (d) or (e) above specifically.

Desirably the hybridising molecules will hybridise to such molecules under stringent hybridisation conditions. One example of stringent hybridisation conditions is where attempted hybridisation is carried out at a temperature of from about 35°C to about 65°C using a salt solution which is about 0.9 molar. However, the skilled person will be able to vary such conditions as appropriate in order to take into account variables such as probe length, base composition, type of ions present, etc.

Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

Typically primers will be at least five nucleotides long and will generally be at least ten nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases, primers of at least thirty or at least thirty-five nucleotides in length may be used.

As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to provide a longer sequence.

In addition to being used as primers and/or probes, hybridising nucleic acid molecules of the present invention can be used as anti-sense molecules to alter the expression of substances of the present invention by binding to complementary nucleic acid molecules. This technique can be used in anti-sense therapy.

A hybridising nucleic acid molecule of the present invention may have a high degree of sequence identity along its length with a nucleic acid molecule within the scope of (a)-(e) above (e.g. at least 50%, at least 75% or at least 90% or 95% sequence identity). As will be appreciated by the skilled person, the higher the sequence identity a given single stranded nucleic acid molecule has with another nucleic acid molecule, the greater the likelihood that it will hybridise to a nucleic acid molecule which is complementary to that other nucleic acid molecule under appropriate conditions.

In view of the foregoing description the skilled person will appreciate that a large number of nucleic acids are within the scope of the present invention. Unless the context indicates otherwise, nucleic acid molecules of the present invention may have one or more of the following characteristics:

- they may be DNA or RNA;
- they may be single or double stranded;
- they may be provided in recombinant form, e.g. covalently linked to a 5' and/or a 3' flanking sequence to provide a molecule which does not occur in nature;
- they may be provided without 5' and/or 3' flanking sequences which normally occur in nature;

- they may be provided in substantially pure form. Thus they may be provided in a form which is substantially free from contaminating proteins and/or from other nucleic acids; and
- they may be provided with introns or without introns (e.g. as cDNA).

A convenient means for detecting/quantifying the polypeptides used in the present invention involves the use of antibodies. Thus, the polypeptides used in the invention also find use in raising antibodies.

In a ninth aspect, the present invention provides an antibody which binds to at least one polypeptide of the invention.

In a tenth aspect, the invention provides the use of an antibody of the invention for screening for and/or diagnosis of a neuropsychiatric or neurological disorder in a subject and/or monitoring the effectiveness of a neuropsychiatric or neurological disorder therapy in a subject. Preferably, the antibody is used for detecting and/or quantifying the amount of a polypeptide of the invention in a biological sample obtained from said subject.

In an eleventh aspect, the present invention provides a method for the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder in a subject, which comprises administering to said subject a therapeutically effective amount of at least one antibody of the invention.

In a twelfth aspect, the present invention provides the use of at least one antibody of the invention in the preparation of a medicament for use in the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder.

Preferred antibodies bind specifically to polypeptides of the present invention so that they can be used to purify and/or inhibit the activity of such polypeptides. The antibodies may be monoclonal or polyclonal.

Thus, the polypeptide used in the invention, its fragments or other derivatives, or analogues thereof, may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanised or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen-binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognise a specific domain of a polypeptide used in the invention, one may assay generated hybridomas for a product which binds to a polypeptide fragment containing such domain. For selection of an antibody that specifically binds a first polypeptide homologue but which does not specifically bind to (or binds less avidly to) a second polypeptide homologue, one can select on the basis of positive binding to the first polypeptide homologue and a lack of binding to (or reduced binding to) the second polypeptide homologue.

For preparation of monoclonal antibodies (mAbs) directed toward a polypeptide used in the invention, any technique which provides for the production of antibody

molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs used in the invention may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilising known technology (PCT/US90/02545).

The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (e.g., human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., U.S. Patent No. 4,816,567; and U.S. Patent No. 4,816397). Humanised antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., U.S. Patent No. 5,585,089).

Chimeric and humanised monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in WO 87/02671; EP-A-184,187; EP-A-171,496; EP-A-173,494; WO 86/01533; U.S. Patent No. 4,816,567; EP-A-125,023; Better *et al.*, 1988, *Science* 240:1041-1043; Liu *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.*, 1987, *Canc. Res.* 47:999-1005; Wood *et al.*, 1985, *Nature* 314:446-449; Shaw *et al.*, 1988, *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, 1985, *Science* 229:1202-1207; Oi *et al.*, 1986, *Bio/Techniques* 4:214; U.S. Patent 5,225,539; Jones *et*

al., 1986, *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.*, 1988, *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunised in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide used in the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harboured by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg & Huszar (1995), *Int. Rev. Immunol.* 13:65-93.

Completely human antibodies which recognise a selected epitope can be generated using a technique referred to as "guided selection". In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognising the same epitope. (Jespers *et al.* (1994) *Biotechnology* 12:899-903).

The antibodies used in the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilised to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labelled antigen or antigen bound or captured to a solid surface or bead. Phage used in these

methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulphide stabilised Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies used in the present invention include those disclosed in Brinkman *et al., J. Immunol. Methods* 182: 41-50 (1995); Ames *et al., J. Immunol. Methods* 184:177-186 (1995); Kettleborough *et al., Eur. J. Immunol.* 24:952-958 (1994); Persic *et al., Gene* 187 9-18 (1997); Burton *et al., Advances in Immunology* 57:191-280 (1994);. PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al., BioTechniques* 12(6):864-869 (1992); and Sawai *et al., AJRI* 34:26-34 (1995); and Better *et al., Science* 240:1041-1043 (1988).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al., Methods in Enzymology* 203:46-88 (1991); Shu *et al., PNAS* 90:7995-7999 (1993); and Skerra *et al., Science* 240:1038-1040 (1988).

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light

chain pairs, where the two chains have different specificities (Milstein *et al.*, 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, 1991, *EMBO J.* 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the

bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details for generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 1986, 121:210.

The invention provides for the use of functionally active fragments, derivatives or analogues of the anti-polypeptide immunoglobulin molecules. "Functionally active" means that the fragment, derivative or analogue is able to elicit anti-anti-idiotype antibodies (i.e., tertiary antibodies) that recognise the same antigen that is recognised by the antibody from which the fragment, derivative or analogue is derived. Specifically, in a preferred embodiment, the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognises the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')2 fragments and Fab fragments. Antibody fragments which recognise specific epitopes may be generated by known techniques. F(ab')2 fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulphide bridges of the F(ab')2 fragments. The invention also provides heavy chain and light chain dimmers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (e.g., as described in U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423-42; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward *et al.*, 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain

polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra *et al.*, 1988, *Science* 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins used in the invention include analogues and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogues of the immunoglobulins include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatisation by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analogue or derivative may contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localisation and activity of the polypeptides used in the invention, e.g., for imaging or radioimaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc. and for radiotherapy.

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression technique.

Recombinant expression of antibodies, or fragments, derivatives or analogues thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesised oligonucleotides (e.g., as described in Kutmeier *et al.*, 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridisable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognises a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunising an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (e.g., as described in Huse *et al.*, 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or

by screening antibody libraries (See, e.g., Clackson *et al.*, 1991, *Nature* 352:624; Hane *et al.*, 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., WO 86/05807; WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulphide bond with an amino acid residue that does not contain a sulphydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, *in vitro* site directed mutagenesis (Hutchinson *et al.*, 1978, *J. Biol. Chem.* 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger *et al.*, 1984, *Nature* 312:604-608; Takeda *et al.*, 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, e.g., humanised antibodies.

Once a nucleic acid encoding an antibody molecule has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the

polypeptides used in the invention by expressing nucleic acid containing the antibody molecule sequences are well known to those skilled in the art.

Once the antibody molecule used in the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g., ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Alternatively, any fusion protein may be readily purified by utilising an antibody specific for the fusion protein being expressed.

In a preferred embodiment, antibodies of the invention or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

Antibodies used in the invention or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications; Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabelled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in U.S. Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

As discussed herein, certain polypeptides, nucleic acid molecules and antibodies find use in the treatment or prophylaxis of a neuropsychiatric or neurological disorder. Thus, in a tenth aspect, the present invention provides a pharmaceutical formulation comprising at least one one polypeptide, nucleic acid molecule or antibody of the invention, together with one or more pharmaceutically acceptable excipients, carriers or diluents.

The medicament will usually be supplied as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form (depending upon the desired method of administering it to a patient).

It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; as powders or granules; as solutions, syrups or

suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions).

Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof.

Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc.

For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions, oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, 3(6):318 (1986).

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For infections of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base. Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas.

Pharmaceutical compositions adapted for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulisers or insufflators.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the polypeptide, nucleic acid or antibody used in of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used. This dosage may be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice.

The invention also provides a polypeptide of the invention, a nucleic acid of the invention or an antibody of the invention for use in medicine.

In view of the relevance of ADPI-41 in neurological and neuropsychiatric disorders, it may be used in screening methods to identify modulators (*e.g.*, agonists or antagonists) of the expression or activity of ADPI-41. Compounds that modulate the expression of ADPI-41 *in vitro* can be identified by comparing the expression of ADPI-41 in cells treated with a test compound to the expression of ADPI-41 in cells treated with a control compound (e.g., saline). Methods for detecting expression of ADPI-41 are known in the art and include measuring the level of ADPI-41 RNA (e.g., by northern blot analysis or RT-PCR) and measuring ADPI-41 protein (e.g., by immunoassay or western blot analysis). Compounds that modulate the activity of ADPI-41 can be identified by comparing the ability of a test compound to agonise or antagonise a function of ADPI-41, for example to regulate the accumulation of iron within mitochondria, to the ability of a control compound (e.g., saline) to inhibit the same function of ADPI-41. Compounds capable of decreasing the accumulation of

iron may be suitable for further development as compounds useful for the treatment of neurological and neuropsychiatric disorders.

Compounds identified *in vitro* that affect the expression or activity of ADPI-41 can be tested *in vivo* in animal models of a neurological or neuropsychiatric disorder, or in subjects having a neurological or neuropsychiatric disorder, to determine their therapeutic efficacy.

In one embodiment of the invention, compounds that modulate (i.e., upregulate or downregulate) the expression and/or activity of ADPI-41 are administered to a subject in need of treatment or for prophylaxis of a neuropsychiatric or neurological disorder. Antibodies that modulate the expression, activity or both the expression and activity of ADPI-41 are suitable for this purpose. In addition, nucleic acids coding for all or a portion of ADPI-41, or nucleic acids complementary to all or a portion of ADPI-41, may be administered. ADPI-41, or fragments of the ADPI-41 polypeptide, may also be administered.

Thus, the invention further provides a method of screening for agents that modulate the expression or activity of a polypeptide of the invention, or the expression of a nucleic acid molecule of the invention, comprising:

comparing the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the presence of an agent with the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the absence of the agent; and

determining whether the agent causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change.

The invention also provides an agent identified by such a method which causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change. Agents found in the above-described screening methods may be

used in the manufacture of a medicament for the treatment of a neuropsychiatric or neurological disorder, or in a method for treating such a disorder.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law. The citation of any reference herein should not be deemed as an admission that such reference is available as prior art to the instant invention.

Example 1: ADPI-41 is differentially expressed in Alzheimer's disease

Briefly, using the 2-D electrophoresis procedure described in WO 98/23950 and WO 01/63293, proteins in a total of 36 brain tissue samples from different areas of the brain (see Table I below) from subjects having Alzheimer's disease and 38 matched control samples were separated by isoelectric focusing, followed by SDS- PAGE and analysed. The gels were stained and digitally imaged and processed. Figure 1 is an image obtained from 2-dimensional electrophoresis of normal brain tissue, which has been annotated to identify ten landmark features, designated BR1 to BR10. Landmark identification was used to determine the pI and MW of features detected in the images. Gels were subsequently cross-matched and a statistical analysis conducted to determine fold changes for each feature in the gels, both overall in Alzheimer's disease as well as on a region-by-region basis.

This analysis revealed one feature, identified as ADPI-41, having an apparent molecular weight of 32806 Da and an apparent pI of 9.84, which was present in reduced amounts in all areas of the brain tested, with the exception of Amygdala. In addition, ADPI-41 has also been identified in other tissue preparations including primary breast biopsy tissue, membrane fractions from prostate, breast and hepatocellular carcinoma and neuronal cell lines.

The results obtained from Alzheimer's disease tissue homogenate are presented in Table I.

Table I

Region	Fold Change	% of Alzheimer's samples in which ADPI-41 was detected	% of control samples in which ADPI-41 was detected
All AD	-1.121	39% (n=36)	37% (n=38)
Entorhinal Cortex	-11.472	67% (n=3)	67% (n=3)
Neocortex	-3.090	33% (n=3)	33% (n=3)
Frontal Cortex	-1.244	44% (n=9)	50% (n=10)
Hippocampus	-1.350	33% (n=18)	31% (n=19)

Example 2: Isolation And Characterisation Of ADPI-41

Proteins were robotically excised and processed to generate tryptic digest peptides as described in WO 98/23950 and WO 01/63293. Tryptic peptides were analyzed by mass spectrometry using a PerSeptive Biosystems Voyager- DETM STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analyzed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a nanoflowTM electrospray Z-spray source. For partial amino acid sequencing and identification of ADPI-41 uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program version v.C.1 (Eng *et al.*, 1994, *J. Am. Soc. Mass Spectrom.* **5**:976-989), and the method described in PCT Application No. PCT/GB01/04034, which is incorporated herein by reference in its entirety. Criteria for database identification included: the cleavage specificity of trypsin, the detection of a suite of a, b and y ions in peptides returned from the database. The database searched was database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible

at http://www.ncbi.nlm.nih.gov/ and also constructed of Expressed Sequence Tags entries (NCBI).

As a result of database searching, the following amino acid sequences of tryptic digest peptides of ADPI-41 were determined from matches to tryptic digest peptides in conceptual translation of ESTs:

BE298534, AI014241, and AV655958: NILLTNEQLESAR.
AA568689, AW796078, AA782417: QAITQVVVSR.
BF126487, BG388906, BG577432: VGIPVTDENGNR

These sequences have a very high similarity to tryptic digest peptides in the mouse sideroflexin 1 protein (from mitochondria, accession number 15147224, accessible at http://www.ncbi.nlm.nih.gov/entrez/).

Example 3: Cloning of ADPI-41 and identification of splice variants

The predicted full length ADPI-41 ORF was amplified by PCR from human brain and liver cDNA libraries, using the following primers:

Sense (F1), SEQ ID NO: 5 - 5′ actgagcgggacctgcgagc 3′

Antisense (R1), SEQ ID NO: 6 - 5′ tccgtaactgggagaacccagg 3′

The following cycling parameters were used: 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72 °C for 30s.

As a result, a protein having the amino acid sequence shown in Figure 2b, and encoded by the DNA sequence shown in Figure 2a was obtained. A Blast search against High Throughput Genomic Sequencing data (http://www.ncbi.nlm.nih.gov/blast) localised the sequence encoding the protein to chromosome 5 clone RP11-606P24 (AC025713).

The DNA sequences encoding the three identified tryptic digest peptides are as follows:

```
aac att ctg tta acc aac gaa caa ctc gag agt gcg aga
Asn Ile Leu Leu Thr Asn Glu Gln Leu Glu Ser Ala Arg

caa gcc atc acg caa gtt gtc gtg tcc agg
Gln Ala Ile Thr Gln Val Val Val Ser Arg
```

and

```
gtt ggc att ccc gtc acg gat gag aat ggg aac cgc
Val Gly Ile Pro Val Thr Asp Glu Asn Gly Asn Arg
```

Using the primers described above, a splice variant was amplified from both brain and liver (sequence ID: 3 and Figure 3a), in addition to the full-length clone. When compared to the genomic sequence, the splice variant is lacking a complete exon such that the reading frame is not maintained in this shorter version, and the translated protein is different after the unspliced exon (sequence ID: 4 and Figure 3b).

A Blast search (http://www.ncbi.nlm.nih.gov/blast) showed the amino acid sequence of ADPI-41 to be 95% identical (305/322) to sideroflexin 1 (Sfxn1 acc# =15147224) (blastp against the nr database, expect = 10, ungapped alignment). Sxfn 1 was recently identified in a mouse model of sideroblastic anaemia (Fleming, *et al.* (2001). *Genes Dev* **15**(6): 652-7.). In this model, the *flexed-tail mouse (f/f)*, an insertion of a single adenine leads to a frameshift and it is predicted that this would lead to the production of a truncated Sxfn protein. In support of this idea, Western blots have confirmed that Sxfn1 is not detected in *f/f* mice.

Sideroblastic anaemia is a group of disorders characterised by an inability to synthesis functional heme (Koc & Harris (1998). *Am J Hematol* **57**(1): 1-6; May & Bishop (1998). *Haematologica* **83**(1): 56-70; Worwood, (1999). *Br Med Bull* **55**(3): 556-67). The iron uptake mechanisms into the mitochondria appear to be normal, but the iron then accumulates rather than being incorporated into heme. This results in the formation of ring sideroblasts – erythrocytes with an abnormal accumulation of iron in their mitochondria.

This is the first demonstration of the presence of human Sxfn protein. Without wishing to be bound by theory, it is thought Sxfn may represent a novel family of eukaryotic transmembrane transporters involved in the transport of a component essential for iron utilisation in and out of the mitochondria. The Sxfn family shows no homology to any other protein of known function. However, within the family there are conserved blocks of amino acid sequence, in particular, an asparagine rich sequence soon after the first predicted transmembrane domain and a conserved four amino acid motif (see blocked portions of Figures 2b and 3b).

This is the first demonstration of the human Sxfn protein and the first indication that it may be associated with the occurrence of Alzheimer's disease. Previous work has demonstrated that there is an accumulation of iron in AD and a possible deregulation of iron transport/homeostasis. In sideroblastic anaemia, it has been suggested that the accumulation of iron in the mitochondria may increase the levels of oxidative stress. Iron is a source of free radicals in via the "Fenton Reaction" in which iron (II) is oxidised to iron (III) resulting in the production of a hydroxyl free radical. There is a great deal of evidence that there is an increase in oxidative damage in Alzheimer's disease. As demonstrated herein, there is a decrease in the expression level of ADPI-41/Sxfn in the brain in Alzheimer's disease patients. This decrease in Sxfn may contribute to the increase in iron accumulation seen in Alzheimer's disease, and thus to the oxidative damage seen.

Several arguments suggest an important role for ADPI-41 as a marker and/or therapeutic target in Alzheimer's disease. An iron dysregulation in Alzheimer's disease has been reported, associated with an accumulation of iron (Smith, *et al.* (1997). *Proc Natl Acad Sci U S A* **94**(18): 9866-8; Smith, *et al.* (2000). *Antioxid Redox Signal* **2**(3): 413-20). This is in agreement with the proposed role of Sxfn in sideroblastic anaemia where a loss of Sxfn protein is associated with an accumulation of iron in mitochondrial of normoblasts. Iron is able to mediate free-radical production via the Fenton Reaction and this may well contribute to the oxidative damage seen throughout the brain in Alzheimer's disease patients (Smith, *et al.* (2000). *Antioxid Redox Signal* **2**(3): 413-20). In addition, we provide the first evidence that the protein undergoes alternative splicing to result in an isoform of 261 amino acids.

Example 4: ADPI-41 expression in a variety of tissue types.

The following example illustrates the detection of fragments of the ADPI-41 polypeptide in biological samples taken from various human tissues or cell lines. The peptides were identified as substantially as described above in Examples 1 and 2, except that each identified sample was substituted for the starting material. Each fragment of Table II corresponds to a fragment found in the sequence of ADPI-41.

Table II

	Tissue Sample									
Peptide Fragment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ANHFFTVTDPR			X							
NILLTNEQLESAR	X	X	X		X	X	X	X	X	
QGIVPPGLTENELWR			X							
YIYDSAFHPDTGEK			X				X	X		
HVSPLIGR			X			X				
VGIPVTDENGNR	X	X	X	X	X	X		X		X
QAITQVVVSR	X	X	X				X	X	X	
IQESHPELR							X	X		

Tissue Sample Key:

1 Brain tissue homogenate from Alzheimer's disease patients
2 Brain tissue homogenate from control subjects
3 Breast carcinoma cell line (membrane fraction)
4 Primary breast cancer tissue samples (membrane fraction)
5 Lymph node samples from breast cancer patients
6 Clinical samples from late stage chronic lymphocytic leukaemia patients
7 Neuroblastoma cell line samples (membrane fraction)
8 Acute monocytic leukaemia cell line samples (membrane fraction)
9 Prostate cancer cell line (membrane fraction)
10 Hepatocellular carcinoma cell line sample (membrane fraction)

Each of the identified fragments came from a peptide having a pI and MW within 10% of ADPI-41.

In addition to the results in Alzheimer's disease tissue and the other tissues listed above, in neuroblastoma cell lines, treatment with a cholinergic agonist substantially alters the expression levels of ADPI-41. Alterations in neurotransmitters are implicated in many neurological and neuropsychiatric diseases.

It is to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

While the invention has been described and illustrated herein by references to the specific embodiments, various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Claims

1. A substantially pure, isolated or recombinant polypeptide which includes the sequence YIYDSAFHPDTGEK.

2. A polypeptide as claimed in claim 1, which includes the sequence: ANHFFTVTDPRNILLTNEQLESARKIVHDYRQGIVPPGLTENELWRAKYIYDSA FHPDTGEKMILIGRMSAQVPMNMTITGCMMTFYRTTPAVLFWQWINQSFNAV VNYTNR.

3. A polypeptide as claimed in claim 1 or claim 2, which:

a) comprises or consists of the amino acid sequence shown in Figure 2b or 3b;

b) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence shown in Figure 2b or 3b; or

c) is a fragment of a polypeptide as defined in a) or b) above, which is at least ten amino acids long.

4. A polypeptide as claimed in claim 1, 2 or 3, which is provided as part of a fusion polypeptide.

5. An isolated or recombinant nucleic acid molecule which encodes a polypeptide as defined in any one of claims 1 to 4.

6. A nucleic acid molecule as claimed in claim 5, which:

a) comprises or consists of the DNA sequence shown in Figure 2a or 3a or its RNA equivalent;

b) has a sequence which is complementary to the sequences of a);

c) has a sequence which codes for the same polypeptide as the sequences of a) or b);

d) has a sequence which shows substantial identity with any of those of a), b) and c); or

e) has a sequence which codes for a derivative or fragment of an amino acid molecule shown either in Figures 2b or 3b.

7. A vector comprising one or more nucleic acid molecules as defined in claim 5 or claim 6.

8. A host cell transformed/transfected with a vector as defined in claim 7.

9. An antibody which binds to a polypeptide as defined in any one of claims 1 to 4.

10. An antibody as claimed in claim 9, which binds specifically to a polypeptide as defined in any one of claims 1 to 4.

11. An antibody as claimed in claim 9 or claim 10 which is monoclonal or polyclonal.

12. An antibody as claimed in claim 9, 10 or 11, which is conjugated to a therapeutic moiety.

13. An antibody as claimed in claim 12, wherein the therapeutic moiety is a second antibody or a fragment or derivative thereof, a cytotoxic agent or a cytokine.

14. A pharmaceutical formulation comprising at least one polypeptide as claimed in claim 1, 2, 3 or 4, a nucleic acid molecule as claimed in claim 5 or claim 6, and/or an antibody as claimed in any one of claims 9 to 13, optionally together with one or more pharmaceutically acceptable excipients, carriers or diluents.

15. A polypeptide as claimed in claim 1, 2, 3 or 4, a nucleic acid molecule as claimed in claim 5 or claim 6, or an antibody as claimed in any one of claims 9 to 13 for use in medicine.

16. A method of screening for and/or diagnosis of a neuropsychiatric or neurological disorder in a subject and/or monitoring the effectiveness of a neuropsychiatric or neurological disorder therapy, which method comprises the step of detecting and/or quantifying the amount of a polypeptide as claimed in claim 1, 2, 3 or 4, and/or a nucleic acid molecule as claimed in claim 5 or claim 6 in a biological sample obtained from said subject.

17. A method as claimed in claim 16, wherein the amount of a polypeptide is detected and/or quantified using an antibody.

18. A method as claimed in claim 17, wherein the antibody is an antibody as claimed in any one of claims 9 to 13.

19. A method for the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder in a subject, which comprises administering to said subject a therapeutically effective amount of at least one polypeptide as claimed in claim 1, 2, 3 or 4, a nucleic acid molecule as claimed in claim 5 or claim 6, and/or an antibody as claimed in any one of claims 9 to 13.

20. The use of at least one polypeptide as claimed in claim 1, 2, 3 or 4, a nucleic acid molecule as claimed in claim 5 or claim 6, and/or an antibody as claimed in any one of claims 9 to 13 in the preparation of a medicament for use in the prophylaxis and/or treatment of a neuropsychiatric or neurological disorder.

21. A method of screening for agents that modulate the expression or activity of a polypeptide as defined in claim 1, 2, 3 or 4, or the expression of a nucleic acid molecule as defined in claim 5 or claim 6, comprising:

comparing the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the presence of an agent with the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the absence of an agent; and

determining whether the agent causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change.

22. An agent identified by the method of claim 21 which causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change.

23. The use of an agent as claimed in claim 22 in the manufacture of a medicament for the treatment of a neuropsychiatric or neurological disorder.

Figure 1

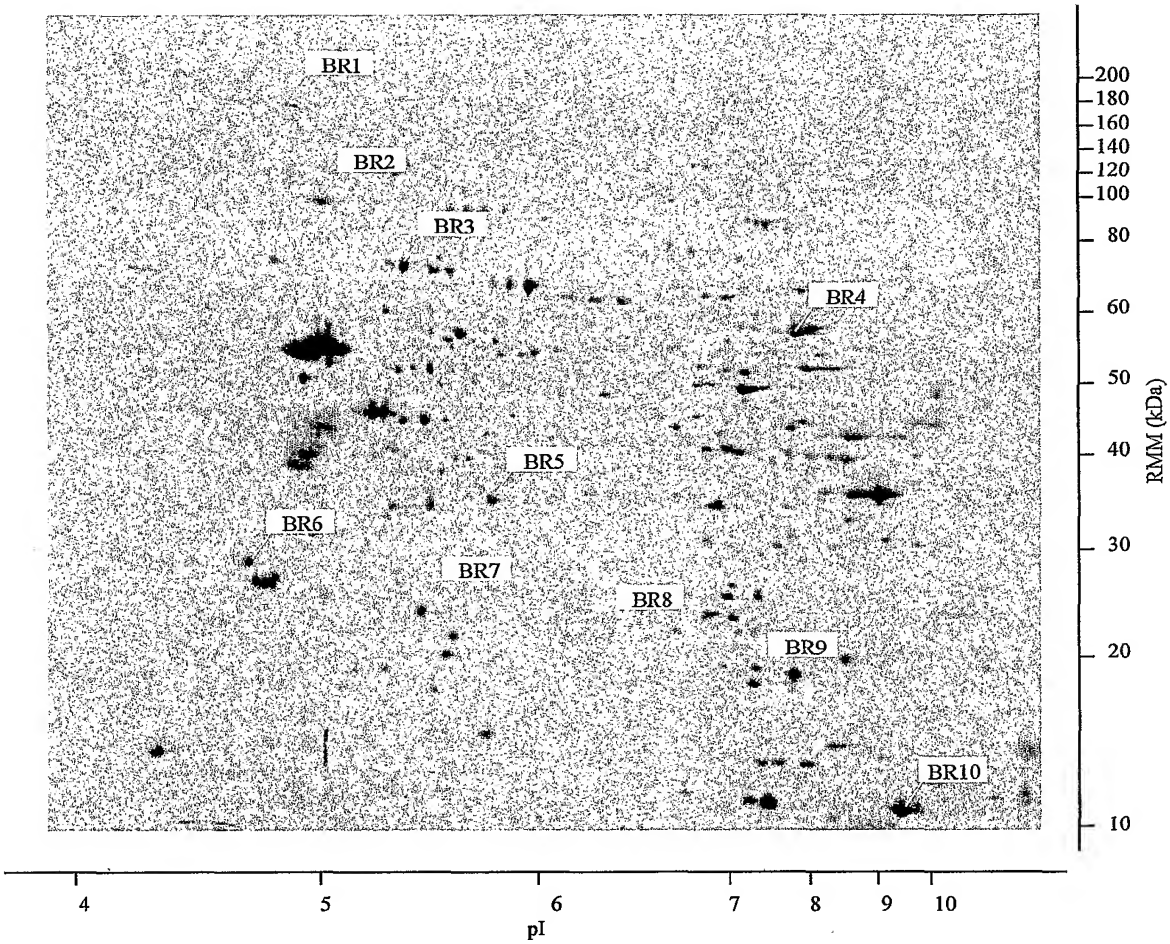
BR1
BR2
BR3
BR4
BR5
BR6
BR7
BR8
BR9
BR10
200
180
160
140
120
100
80
60
50
40
30
20
10
RMM (kDa)
4
5
6
7
8
9
10
pI

Figure 2a

```
   1  gtccgggacc atgtctggag aactaccacc aaacattaac atcaaggaac
  51  ctcgatggga tcaaagcact ttcattggac gagccaatca tttcttcact
 101  gtaactgacc ccaggaacat tctgttaacc aacgaacaac tcgagagtgc
 151  gagaaaaata gtacatgatt acaggcaagg aattgttcct cctggtctta
 201  cagaaaatga attgtggaga gcaaagtaca tctatgattc agcttttcat
 251  cctgacactg gtgagaagat gattttgata ggaagaatgt cagcccaggt
 301  tcccatgaac atgaccatca caggttgtat gatgacgttt tacaggacta
 351  cgccggctgt gctgttctgg cagtggatta accagtcctt caatgccgtc
 401  gtcaattaca ccaacagaag tggagacgca cccctcactg tcaatgagtt
 451  gggaacagct tacgtttctg caacaactgg tgccgtagca acagctctag
 501  gactcaatgc attgaccaag catgtctcac cactgatagg acgttttgtt
 551  ccctttgctg ccgtagctgc tgctaattgc attaatattc cattaatgag
 601  gcaaagggaa ctcaaagttg gcattcccgt cacggatgag aatgggaacc
 651  gcttggggga gtcggcgaac gctgcgaaac aagccatcac gcaagttgtc
 701  gtgtccagga ttctcatggc agcccctggc atggccatcc ctccattcat
 751  tatgaacact ttggaaaaga aagccttttt gaagaggttc ccatggatga
 801  gtgcacccat tcaagttggg ttagttggct tctgtttggt gtttgctaca
 851  cccctgtgtt gtgccctgtt tcctcagaaa agttccatgt ctgtgacaag
 901  cttggaggcc gagttgcaag ctaagatcca agagagccat cctgaattgc
 951  gacgcgtgta cttcaataag ggattgtaaa gcagggagga aacctctgca
1001  gctcattctg ccactgcaaa gctggtgtag ccatgctggt gagaaaaatc
1051  ctgttcaacc tgggttctcc cagttacgga aagggcgaat tcgcggccgc
1101  taattcgatt cgccctatag nagtngtaac antc
```

Figure 2b

```
   1  MSGELPPNIN IKEPRWDQST FIGRANHFFT VTDPRNILLT NEQLESARKI
  51  VHDYRQGIVP PGLTENELWR AKYIYDSAFH PDTGEKMILI GRMSAQVPMN
 101  MTIIGCMMTF YRTTPAVIFW QWINQSFNAV VNYTNRSGDA PLTVNELGTA
 151  YVSATTGAVA TALGLNALTK HVSPLIGRFV PFAAVAAANC INTPLMRQRE
 201  LKVGIPVTDE NGNRLGESAN AAKQAITQVV VSRTLMAAPG MAIPPFIMNT
 251  LEKKAFLKRF PWMSAPIQVG LVGFCLVFAI PLCCALFPQK SSMSVTSLEA
 301  ELQAKIQESH PELRRVYFNK GL*
```

Figure 3a

```
1     gtccgggacc atgtctggag aactaccacc aaacattaac atcaaggaac
51    ctcgatggga tcaaagcact ttcattggac gagccaatca tttcttcact
101   gtaactgacc ccaggaacat tctgttaacc aacgaacaac tcgagagtgc
151   gagaaaaata gtacatgatt acaggcaagg aattgttcct cctggtctta
201   cagaaaatga attgtggaga gcaaagtaca tctatgattc agcttttcat
251   cctgacactg gtgagaagat gattttgata ggaagaatgt cagcccaggt
301   tcccatgaac atgaccatca caggttgtat gatgacgttt tacaggacta
351   cgccggctgt gctgttctgg cagtggatta accagtcctt caatgccgtc
401   gtcaattaca ccaacagaag tggagacgca cccctcactg tcaatgagtt
451   gggaacagct tacgtttctg taacaactgg tgccgtagca acagctctag
501   gactcaatgc attgaccaag catgtctcac cactgatagg acgttttgtt
551   ccctttgctg ccgtagctgc tgctaattgc attaatattc cattaatgag
601   gcaaagccat ccctccattc attatgaaca ctttggaaaa gaaagccttt
651   ttgaagaggt tcccatggat gagtgcaccc attcaagttg ggttagttgg
701   cttctgtttg gtgtttgcta cacccctgtg ttgtgccctg tttcctcaga
751   aaagttccat gtctgtgaca agcttggagg ccgagttgca agctaagatc
801   caagagagcc atcctgaatt gcgacgcgtg tacttcaata agggattgta
851   aagcagggag gaaacctctg cagctcattc tgccactgca aagctggtgt
901   agccatgctg gtgagaaaaa tcctgttcaa cctgggttct cccagttang
951   gaaagggcga attcgcggcc gctgattcna ttac
```

Figure 3b

```
1     MSGELPPNIN IKEPRWDQST FIGRANHFFT VTDPRNILLT NEQLESARKI
51    VHDYRQGIVP PGLTENELWR AKYIYDSAFH PDTGEKMILI GRMSAQVPMN
101   MTITGCMMTF YRTTPAVLFW QWINQSFNAV VNYTNRSGDA PLTVNELGTA
151   YVSVTTGAVA TALGLNALTK HVSPLIGRFV PFAAVAAANC INIPLMRQSH
201   PSIHYEHFGK ESLFEEVPMD ECTHSSWVSW LLFGVCYTPV LCPVSSEKFH
251   VCDKLGGRVA S*
```